BIOPHYSICAL RESEARCH LABORATORY

FIFTEENTH ANNUAL REPORT

CARNEGIE-MELLON UNIVERSITY
PITTSBURGH, PENNSYLVANIA

Jerome J. Wolken

June 30, 1970

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I. INTRODUCTION

The Biophysical Research Laboratory was established in 1953, with the aim of initiating a long-range research program in Photobiology.

The principal research efforts of the Laboratory for the past sixteen years have been directed toward the comparative study of photoreceptor structures for photosynthesis and vision, and how their molecular structure and chemistry are related to their physiological function, as energy transfer devices.

The conversion of one form of energy to another, as light energy to chemical and electrical energy in the physiological process, is of fundamental importance to our understanding of life.

Our approach to the understanding of the energetics of living systems has been through the following methods: (1) A quantitative study of the excitatory forces, the stimuli; (2) a comparative study of the molecular structure of the photoreceptors; (3) a chemical study of the photoreceptor pigments, their biosynthesis and structural identification; (4) a study of the photochemistry of the photoreceptor pigment complexes; (5) a search for model living cells to study these phenomena; and (6) a study of physical, mechanical, chemical and electronic model systems that have behavioral properties analogous to photoreceptors.

In these Annual Reports of the Biophysical Research Laboratory, I-XV, 1953 to 1970, the year-by-year experimental findings are summarized in order to put together the bits of information gathered. In addition, these Reports indicate the experimental tools, the personnel responsible for the research, the philosophy and environment in which the research is carried out.

II. BIOPHYSICAL RESEARCH LABORATORY STAFF, 1969-1970

JEROME J. WOLKEN	B.S., M.S., Ph.D., University of Pittsburgh; Principal Investigator; Professor of Biophysics.
GERALD J. GALLIK	B.S., University of Pittsburgh; M.S., Carnegie-Mellon University; Research Biologist.
ROBERT G. FLORIDA	B.S., University of Pittsburgh; Research Biologist; December, 1958 to March, 1970.
MOHAMED A. ALI	B.S., M.Sc., Presidency College, University of Madras; Ph.D., University of British Columbia; Visiting Research Fellow from University of Montreal, January, 1969 to September, 1969.
MAHENDRA R. VORA	B.Sc., M.Sc., Ph.D., University of Bombay; Visiting Research Fellow, October, 1966 to September, 1969.
PERVEZ J. DUBASH	B.Sc. and Ph.D., University of Bombay; Visiting Research Fellow from University of Bombay, January, 1969 to April, 1970.
TAMOTSU OOTAKI	B.S., Yamagata University; M.S., Ph.D., Nagoya University, Japan; Visiting Research Fellow, July, 1970.
OLIVER J. BASHOR, JR.	Technician.
ARLENE R. MANN	B.A., M.A.T., University of Pittsburgh; Secretary/Editorial Assistant.

ASSOCIATES

RICHARD A. ELCO	B.S., M.S., Ph.D., Carnegie Institute of Technology; Assistant Professor, Electrical Engineering, Carnegie-Mellon University; October, 1969 to May, 1970.
HERBERT REITBOECK	B.S., M.S., Ph.D., Vienna Institute of Technology; Ph.D., University of Frankfurt; Westinghouse Research and Development Center, Senior Engineer.

GRADUATE STUDENTS AND UNDERGRADUATE STUDENT ASSISTANTS

EDWARD L. MOROFSKY	B.S., M.S., Carnegie-Mellon University; Biotechnology.
ALFRED PERLIN	B.S., M.S., Technion, Israel; Biotechnology.
ALOIS A. LANGER	B.S., Massachusetts Institute of Technology; M.S., Carnegie-Mellon University; Electrical Engineering.
WAYNE HAMMOND	B.S., Carnegie-Mellon University.
A. JONATHAN WOLKEN	Dartmouth College.

III. EDUCATIONAL ASPECTS

The Biophysical Research Laboratory plays a part in the Biological Sciences at Carnegie-Mellon University. Research opportunities are available for a number of graduate students and research fellows. The Laboratory also serves for interdisciplinary training and research with other departments in the University. Because Carnegie-Mellon University is geographically close to Eye and Ear Hospital and the University of Pittsburgh, it is possible to continue our cooperative research programs in vision and physiology.

Seminars and tutorials for graduate students are organized in close association with the research of the Biophysical Research Laboratory. In addition to courses in Biophysics (S/GS 557-558), Dr. Wolken has taught a course, Science Systems (A-596), for non-science majors. The Laboratory staff has supervised graduate research (GS/560) and undergraduate investigative topics, instructed students in biophysical tools and methods, and research projects for pre-college students. It is hoped that these programs will foster an interest in research and that the environment of the Laboratory will encourage an enthusiasm toward creative investigation at a crucial stage in the development of scientific careers.

Opportunities and encouragement are given to the research staff of the Laboratory to further their own studies and to work at other research laboratories or universities for periods of time to broaden their own scope.

Since the establishment of the Laboratory, the granting of post-doctoral research fellowships has been a continuing policy. Fellowships have been provided for visiting research scholars and medical students through the generosity of the Scaife Family of Pittsburgh. The research training program effectiveness is undergoing changes, as research grants are being curtailed. It is unfortunate, for this primarily affects our support to graduate students and Visiting Research Fellows. These Fellows help provide the stimulating environment so necessary for productive research.

IV. THE LABORATORY

The Biophysical Research Laboratory occupies 5,000 square feet of well-equipped laboratories -- for biochemistry, biophysics, physical chemistry, electrophysiology, electron microscopy, electron optics, microbiology, magnetics, histology and tissue culture.

These research laboratories include equipment for performing preparative and analytical ultracentrifugation, electron microscopy, spectroscopy, optical diffraction, electrophoresis, ionizing radiation, magnetics and electronics. To service the laboratories there is an experimental instrument shop, photographic darkrooms, greenhouse, specialized library and offices.

In addition, the Laboratory staff has available the use of the Hunt Botanical Library, Carnegie-Mellon University, the natural history collections of the Carnegie Museum, the Phipps Conservatory of Pittsburgh for botanical material and the laboratories of Eye and Ear Hospital, University of Pittsburgh Medical Center, for cooperative research.

V. RESEARCH PROGRESS

Life was initiated under very special conditions -- conditions which made evolution possible, perhaps the most crucial of which was the presence of light. Nature is telling us something about all light-catalyzed reactions; therefore, we are searching carefully into the subtleties of these photobiological phenomena in order to understand them.

In our studies of photoreceptors, from the protozoa to coelenterates, flatworms, roundworms and segmented worms, we find a variety of photoreceptor structures ranging from eyespots to photosensory cells to simple "pin-hole" eyes. In arthropods and molluscs we find, in addition to these more primitive receptors, imaging compound eyes and refracting eyes. We can now see that in the course of evolution invertebrates have used every known optical device for light detection and image formation (refer to Figure 18).

The photoreceptors that have evolved to capture light energy -- the chloroplast for photosynthesis and the retinal rods and cones of the eye for vision -- are all highly ordered structures. As in a crystal, they consist of a lattice of repeat units of about 200 Å spacing (refer to Figures 7, 10-13).

The importance of these structures to photoexcitation, and the kinds of photopigments as well as their molecular structure within these photoreceptors are of considerable interest to us.

A. Photosensory Cells

In search of relatively simple living cells as models to study the conplex phenomena of photosensitivity in nature, and to understand the process, we have pursued our study of the protozoan algal flagellate, Euglena.

> "How a nerve comes to be sensitive to light, hardly concerns us more than how life itself originated; but I may remark that, as some of the lowest organisms, in which nerves cannot be detected, are capable of perceiving light, it does not seem impossible that certain sensitive elements in their sarcode should become aggregated and develop into nerves, endowed with this special sensibility" -- Charles Darwin, 1859.

1. Protozoan Photoreceptor: Eyespot-Flagellum. J. J. Wolken.

How the first photoreceptor evolved is not known; but there is some basis for the suggestion that it may have been an adaptation by a cell for photosynthesis similar to that found in photosynthetic bacteria with chromatophores containing bacterial chlorophyll. Another speculation is that photoreceptors may have been the result of differentiation of flagellar processes of a cell in which a photosensitive pigment became attached to or somehow became associated with a flagellum. If so, this would provide a means for the cell to search for light of the appropriate intensity and wavelengths for photosynthesis. Examples of just such a close association can be seen in the protozoan flagellates; in Chlamydomonas, the eyespot is found within the chloroplast, and in Euglena the eyespot is associated with the flagellum.

In search of answers to how photoreceptor systems developed, how they are structured for light capture, and how they function as an integrated system, it seemed to me that some of the answers would be found among the protozoans that border between plants and animals, as in the unicellular protozoan flagellate, Euglena.

What is the photoreceptor molecule? Euglena gracilis synthesizes three main carotenoids: β-carotene, lutein, and neoxanthin. Lutein was found to be the major pigment comprising 80 per cent of the total. The two pigments which persist in dark-grown euglenas were found to be lutein and β-carotene.

In attempts to identify the Euglena photoreceptor eyespot pigment by chromatography, 80 per cent of the carotenoids were indicated to be lutein, 11 per cent β-carotene, 7 per cent neoxanthin and a small amount of γ-carotene, crytoxanthin, echinenone, keto-carotenoids, euglenanone and hydroxyechinenone. Astaxanthin or astacene was not found.

Assuming that the eyespot is a mixture of carotenoids, for example, β-carotene, lutein and neoxanthin, the absorption peaks would lie within the range 415 to 475 nm. When these pigments are dissolved in carbon disulfide, a nonpolar solvent, the absorption peaks range from 450 to 510 nm. These or similar carotenoids could therefore account for the absorption peaks in the 450 to 510 nm region as found in the phototactic action spectrum for Euglena.

To circumvent the difficulties of isolating the eyespot granules and extraction, the microspectrophotometer M-5 was used to obtain *in situ* absorption spectra from single *Euglena* eyespots of $2\mu^2$ areas. These data showed that the eyespot has a broad absorption band from 430 to 520 nm. Recent studies of absorption spectra of the eyespot show that the peaks lie near 430, 465 and 495 nm, and near 350 and 270 nm in the ultraviolet (Figure 1a). Spectra closer to the base of the flagellum (near the paraflagellar body) show absorption peaks about 440 and 490 nm (Figure 1b). The flagella do not show any peaks in the visible (Figure 1c). In the heat-bleached *Euglena* mutant which lacks chloroplasts, the eyespot spectrum shows absorption peaks near 430, 465 and 510 nm, and in the ultraviolet at 340 nm (Figure 2a). This spectrum resembles in some respects that of a flavin semiquinone, Figure 2b.

When light-grown euglena are dark-adapted for about one hour and mounted on the cold stage (5°C) of the microspectrophotometer, the absorption spectrum obtained (Figure 3a) is similar to that found for the eyespot in Figures 1a and 2a. When the same area is illuminated with strong white light for one to five minutes, the major absorption peak around 490 nm bleaches, shifting its major peak to 440 nm (Figure 3b). This absorption peak at 490 nm is close to the peak found for the phototaxis action spectrum, and has similarities to bleaching of the visual pigment rhodopsin.

Interpretation of the spectra to establish the identity of the photoreceptor pigments in the eyespot is, however, extremely difficult. Action spectra indicate that the eyespot is a shading device for a photosensitive region located at the base of the flagellum and that the photosensitive pigment resembles a flavoprotein with absorption peaks about 370 and 460 nm (see Figure 4, that of riboflavin).

If a flavoprotein is the photoreceptor molecule, then an analysis of the *Euglena* flavins would give us some idea of its concentration. Analysis of the total flavins showed that for both light- and dark-grown *Euglena gracilis*, there were of the order 10^{12} flavin molecules per cell. This is more than sufficient when compared to the number of visual

pigment rhodopsin molecules in the photoreceptors of all eyes, which contain from 10^6 to 10^9 rhodopsin molecules. However, the action spectra and the absorption spectra of the eyespot do not rule out that a carotenoid may be the photoreceptor molecule. These data support the possibility that there are two pigments, a carotenoid and a flavoprotein, in which one is the primary photoreceptor pigment and the other an accessory or screening pigment in the process.

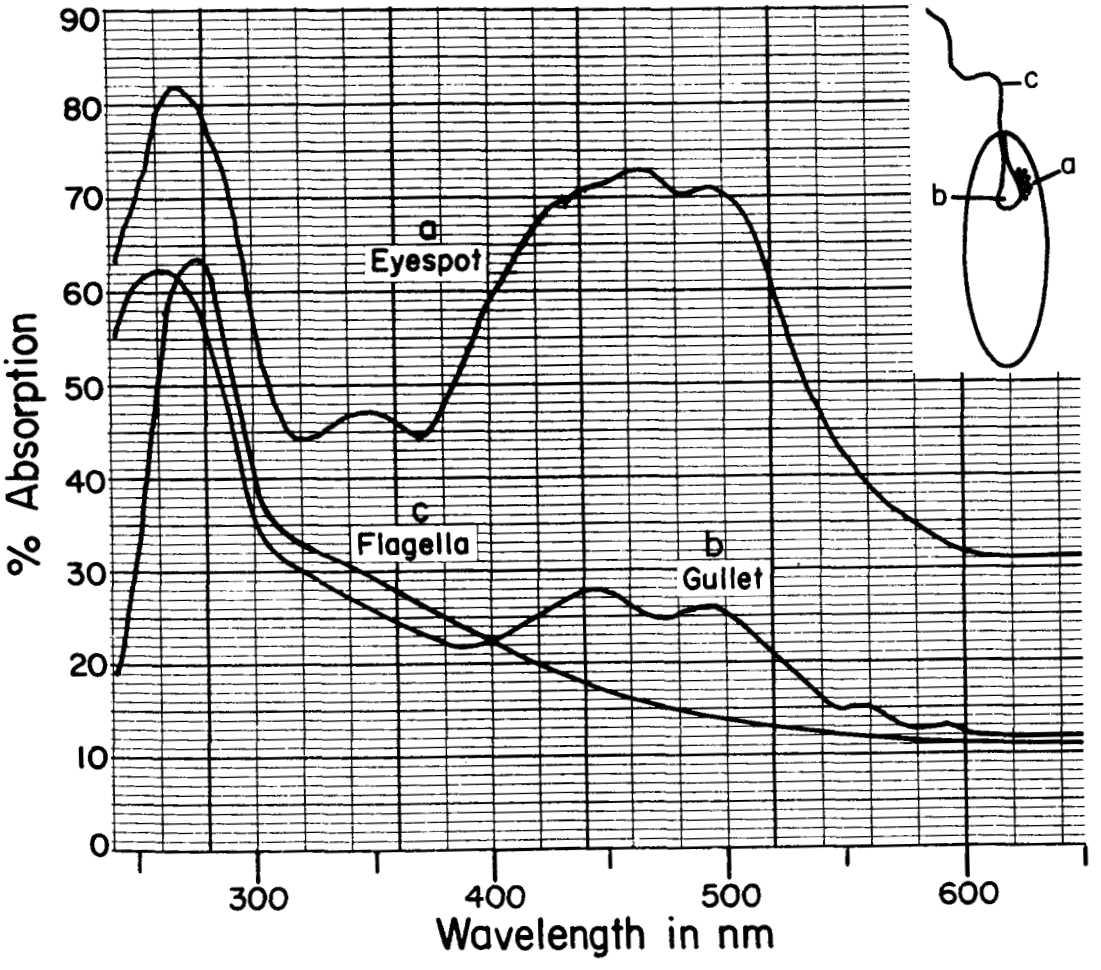


FIGURE 1. Absorption spectra, obtained by microspectrophotometry, of (a) eyespot, (b) gullet, and (c) flagella. Light-grown Euglena gracilis.

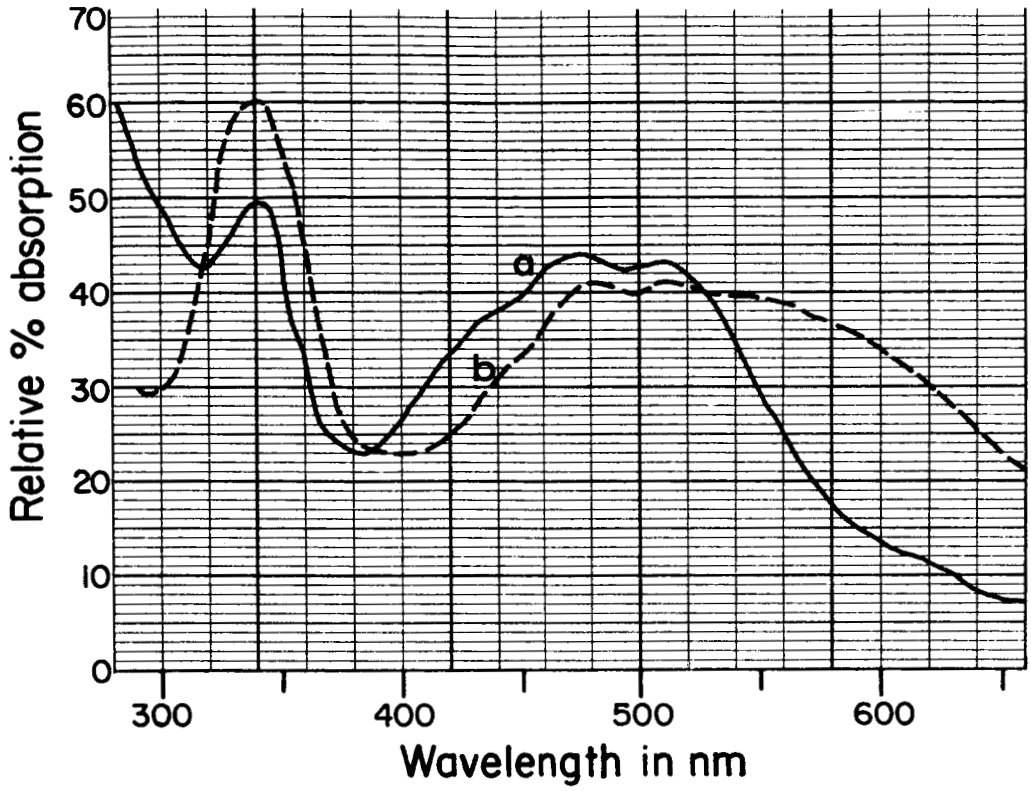


FIGURE 2. Eyespot absorption spectrum (a) of heat-bleached Euglena, obtained by microspectrophotometry, compared to (b) flavin semiquinone, pH 5.1.

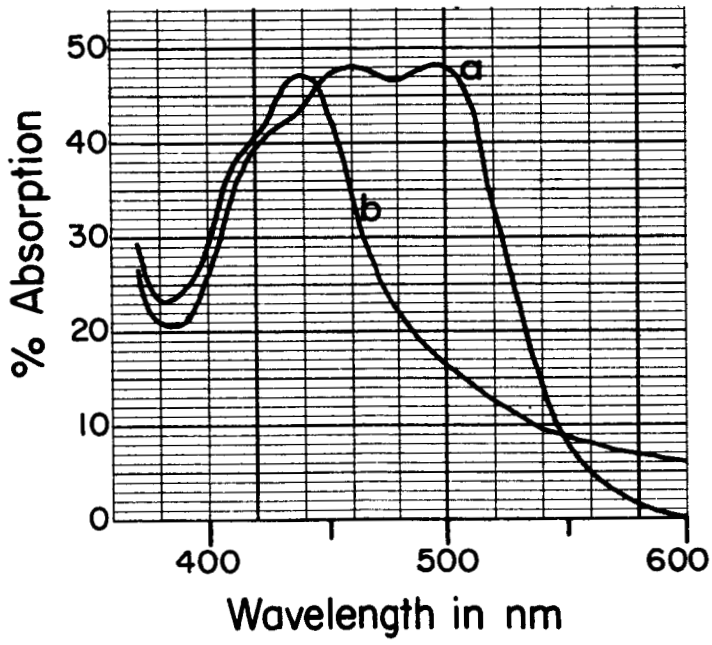


FIGURE 3. Eyespot absorption spectrum, obtained by microspectrophotometry (a) after dark-adaptation for 1 hour and (b) the same eyespot after 5 minutes of white light.

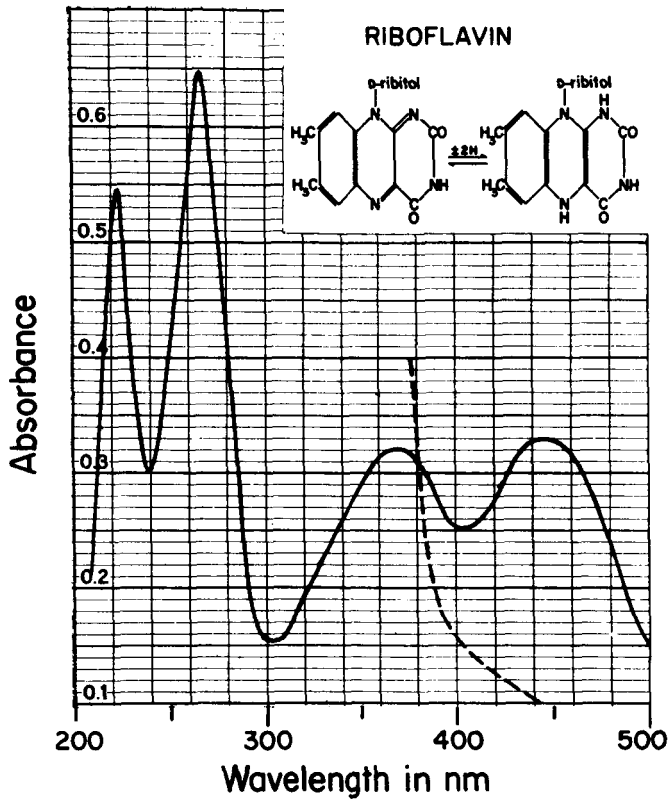


FIGURE 4. Riboflavin. The molecular structure and the absorption spectrum of the oxidized form; when reduced with dithionite (----).

Another cell which we have been investigating is the fungus, Phycomyces blakesleeanus.

2. Phycomyces.

Phycomyces blakesleeanus has long been the object of research concerned with the sensitivity of the sporangiophore to light as measured by the phototropic response and by the light growth response. Light is not essential for growth but functions as a signal to alter the growth in either space or time. To date, however, no photoreceptor structure has been identified and it is not known what pigment or pigment system is responsible for these light responses.

Action spectra show absorption peaks at or near 280, 365-385, 420-425, 445-455 and 475-485 nm. The similarity of the action spectrum to the absorption spectrum of β-carotene in the visible part of the spectrum indicates that it is one of the light receptor molecules. However, the

absorption peaks in the ultraviolet near 280 and 370 nm of the action spectrum and in the visible at 450 nm suggest a flavin or flavoprotein to be one of the photoreceptor molecules (Figures 2b and 4).

To learn more about this phenomenon and whether the action spectra have meaning, the following studies are in progress: electron microscopy of the sporangiophore in search of a photoreceptor structure; differential solvent extraction of the sporangiophore in attempts to isolate a pigment or pigment system; microspectrophotometry to obtain absorption spectra in the phototropic growth-zone; and isolation and characterization of the crystals.

a. The Flavins of Phycomyces. A. J. Wolken.

Neither the action spectra nor the absorption spectra of the growing zone of Phycomyces have been sufficiently informative to clearly identify the primary photoreceptor molecules. Both are ambiguous enough to allow supporting interpretation for either a carotene or a flavin photoreceptor molecule. Quantitative studies of sporangiophore extracts showed that the wild-type Phycomyces contained an extremely large amount of carotenoid, most of which was identified as β-carotene.

In the midst of all this, it would seem that the flavins were neglected. No quantitative work was done to establish either the total amount or the identity of the individual flavins present in the sporangiophore. More recent evidence, however, indicates that the flavins are more deeply involved in the photoreceptor process. For example, microspectrophotometry of Stage IV sporangiophores showed what appeared to be a reduced flavin spectrum beginning in the lower portion of the growing zone and extending down to the base of the sporangiophore. From these spectra, it was clear that Phycomyces sporangiophores contained a large amount of flavins. What was not known was the total amount of flavin present in a sporangiophore, the identity of the individual flavins involved, and the amount each compound contributed to the whole.

Phycomyces blakesleeanus, both wild-type and car-10 strains, were grown on Sabaraud potato-dextrose agar in disposable plastic petri dishes, at 22°C and a humidity near 50%. From the time of innoculation all cultures were kept in continuous fluorescent light of 30 foot

candles intensity. At the end of four days of growth the sporangiophores were harvested by plucking them from the mycelium, after which the petri dishes were replaced in the growth room to allow a new crop of sporangiophores to mature. At the end of the fifth day of growth these "cut back" cultures were harvested again. Immediately after harvesting, the sporangiophores were carefully weighed and placed in the deep freeze (-20°C) in a light-tight container.

For the total flavin analysis, the lumiflavin fluorescence method as described by Yagi (Yagi, K. Chemical Determination of Flavins, In Methods of Biochemical Analysis, Vol. X, David Glick, ed., John Wiley and Sons, Inc., 1962) was employed. For the individual flavin analysis the sample was prepared using Yagi's method with a few modifications. The essential steps used for extraction of the flavins for both total and individual analysis are as follows:

(1) Samples were cut into small pieces. (2) 5 ml of distilled water was added at 80°C, the mixture was heated for 5 minutes at 80°C and allowed to cool. (3) The mixture was homogenized with mortar and pestle and sonicated for 2 minutes. (4) 5 ml of .5% trypsin solution pH 8 was added. A thin layer of toluene was placed on top of the mixture, and this was incubated for 10 hours at 37°C. (5) The mixture was diluted to a total volume of 25 ml, warmed at 80°C for 15 minutes, and allowed to cool. All of the remaining steps in the total flavin analysis followed the method given by Yagi.

The fluorescence of the flavin-containing chloroform layers was measured with a Model 11- Turner Flourometer. All chromatography was done using Eastman chromatogram sheets #6060 (Distillation Products Industries, Rochester, N.Y.), which were thin layers of silica gel with fluorescent indicator. Detection of the developed spots was accomplished by irradiation with a General Electric H85-C3 source in combination with a UV filter.

TABLE I

Total Flavins (Calculated as Riboflavin).

	Total wet weight in grams of sporangiophores used in preparation.	Estimates of total flavins (μg flavin/ g wet weight of sporangiophore).	Number of molecules of flavin per sporangiophore. $\times 10^{12}$
Wild Type (average height of sporangiophore ~8 cm.)	3.47 4.41 11.12	8.64 3.44 5.82	22.10 8.03 10.50
Albino car-10 (average height of sporangiophore ~4 cm.)	5.72	1.78	4.56

These results in Table I indicate that an extremely large amount of flavin is present in the sporangiophores, from 10^{12} to 10^{13} molecules per sporangiophore. Certainly there is more than enough in each sporangiophore to act as the primary photoreceptor -- at least 10^{3} times the necessary number of molecules if you consider the upper limit of photoreceptor molecules per receptor to approach 10^{9}.

What can be said about the relative amounts of the various flavins present in the sporangiophores? By developing the wild-type flavin sample in a bath of n-butanol/acetic acid/H_2O: 4/1/1 for 3 hours, having taken care to expose it to a minimum of light, a green-fluorescing main band was evident with R_f values from .387 to .550. One-dimensional chromatography did not permit further separation of the compounds in this band, so it is likely that more than one flavin was present. In addition, there were fainter bands present, one with an R_f value .734 which fluoresced blue, and a second band of R_f value .037 which fluoresced green.

Establishing the identity of these bands poses a more difficult problem than measuring their R_f values. The main band from ~.38 to ~.55 could indicate a number of flavins; for example, riboflavin or lumiflavin, both of which fluoresce green and gave R_f values similar to those of the main band. The blue band with R_f=.734 would appear to be lumichrome, while

the last band with R_f=.037 would appear to be FAD (Flavin adenine dinucleotide). On one chromatogram a very faint green-fluorescing band appeared which satisfied the R_f criteria for FMN (Flavin mononucleotide).

Chromatography of the albino-10 flavin samples showed less variation. Samples developed with n-butanol/acetic acid/H_2O:4/4/1 for 3 hours yielded a major band with R_f values ranging from .400 to .525, which falls near the value obtained for pure riboflavin (.50 to .55). However, this discrepancy might be explained by the fact that when the standard samples were run, the room temperature was closer to 18 or 20°C. Also, on these chromatograms a faint green area remained close to the origin with an R_f value from .036 to .078. This last band might be interpreted as FMN.

In an effort to produce more dependable results I designed a two-dimensional chromatography development using first n-butanol/n-propanol/H_2O:2/2/1 and then 5% Na_2PO_4. From references run with each solvent system I was able to identify a faint band which fell slightly short of pure riboflavin with an R_f in the first solvent system from .476 to .500. Pure riboflavin gave R_f values from .516 to .566. The butanol/propanol/H_2O system also separated out a blue-fluorescing compound with R_f values from .122 to .175. This compound appeared to fit the requirements of lumichrome. The first solvent also left a highly fluorescent area just beyond the origin, with R_f values less than .10, containing FAD and FMN. The second phase of the chromatography was designed to separate as closely as possible the compounds FAD and FMN, but in this respect it failed; separation remained incomplete and the spot produced was too large and diffuse for quantitative measurements. Nevertheless, the results from the second stage of chromatography (using 5% Na_2HPO_4) indicated that FMN and/or FAD are definitely present in the sporangiophore.

The following three compounds, then, appear to be present in the sporangiophore: FMN and/or FAD, lumichrome, and riboflavin.

These flavins are present in both the wild-type and the albino car-10. Both the wild-type and the car-10 contain close to 10^{13} molecules

of flavin per sporangiophore if the molecular weight of riboflavin is used in the calculation.

How reasonable are these assertions about the sporangiophore? Indeed, it would seem unlikely to find the riboflavin molecule intact and in its pure state; yet the presence of lumichrome greatly enhances its plausibility. Microspectrophotometry of the growing zone showed a shift in spectral characteristic from a typical carotenoid spectrum to a typical reduced flavin spectrum. Speculation that this reduced flavin is in fact lumichrome is supported by the finding of lumichrome in the sporangiophore.

b. Isolation of Phycomyces Crystals. G. J. Gallik, P. J. Dubash and J. J. Wolken.

Phycomyces blakesleeanus wild (-) were grown for 8-10 days in a 3 cm layer of Sabouraud's dextrose agar in a 35 x 20 cm pyrex dish under constant illumination at 22°C at 35% relative humidity. Sporangiophores were periodically examined during their growth phase, under a polarizing microscope, for the presence of refractile crystalline inclusions.

The distribution and frequency of occurrence of crystals in Phycomyces sporangiophores varied with maturation, aging, type of strain and conditions of growth.

Polarized light microscopy showed crystalline birefringent microbodies in the wild (-) strain as well as the albino car-10 strain (Figure 5). The crystals were chiefly located in the growth zone 2-5 mm. below the sporangium where the photoreceptor is believed to lie. In Phycomyces grown in total darkness, only a few crystals were observed in the growth zone of the wild (-) strain after 96 hours, and none were detected in the car-10 strain. Grown in the light for 8 days, the wild (-) strain develops crystals throughout the entire length of the sporangiophore.

Crystals were isolated by sucrose gradient centrifugation. A good yield of crystals was obtained from 8 day old cultures, when the sporangiophores were plucked with a forceps and transferred to a 0.1 M PO_4 buffer pH 7.0. The mixture was then sonicated in the cold

with a Bronson Sonifier at 20,000 cps. for 3 minutes, layered over 60% sucrose solution and centrifuged at 78,480 x g for 20 minutes in a Beckman model L preparative centrifuge. This yielded a crude crystal fraction at the sucrose interface, which was removed with a syringe into another tube, washed twice with distilled water and an aqueous suspension was then layered on a 40% sucrose solution. This was again centrifuged at 78,480 x g for 20 minutes, the crystal fraction was removed and washed as before, layered on a 25% sucrose solution and centrifuged at 54,500 x g for 15 minutes to obtain a relatively pure crystal fraction free of insoluble contaminants. The fraction having a density of 1.1 was washed and resuspended in water and stored in the deep freeze to be used for chemical analysis.

Gross analysis of these crystals showed them to be composed primarily of protein with small amounts of lipids and carotenes with no traces of nucleic acids and flavins. The high protein content of the crystals is attributable to its enzymic nature, the fraction having appreciable acid phosphatase and catalase activity. There was a faintly positive reaction for Fe^{3+}, evidently of catalase origin. These findings are summarized in Table II.

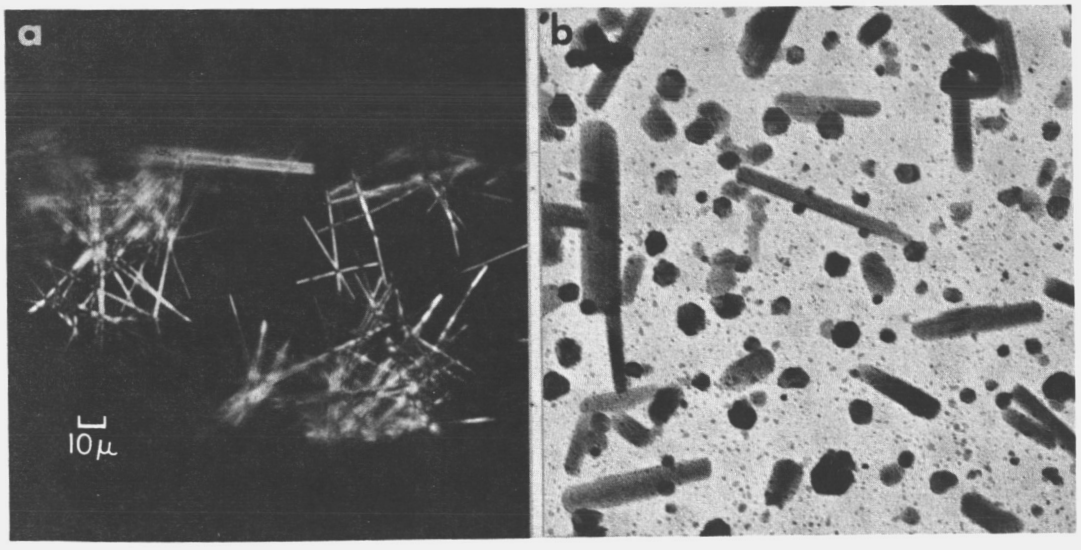


FIGURE 5. (a) Light micrograph of Phycomyces crystals. (b) Electronmicrograph plate of Phycomyces crystals.

TABLE II

Phycomyces blakesleeanus, wild-type (-)

Crystals (density 1.1) isolated from 8 day sporangiophore by sucrose gradient centrifugation.

Analysis (in relative concentrations)

Protein	++++
catalase	+
acid phosphatase	+
peroxidase	-?
Lipids	++
Carotenes	+
Flavins	-
DNA	-
RNA	-
Indole	-?
Iron (Fe^{++})	+-

The protein concentration is almost 90 per cent and the remaining 10 per cent is lipid. Our analysis is summarized in Table III.

TABLE III

Phycomyces blakesleeanus, wild-type (-)

Crystals (density 1.1) isolated from 8 day sporangiophores, by sucrose gradient centrifugation and resuspended in 10 ml. distilled water.

Analysis

Fraction	Concentration ug/ml.	%
Protein	3000	88.9
Lipid (as fatty acids)	375	11.0
Carotenes (β-carotene)	3.9	0.1

Microspectrophotometry of the crystals within a sporangiophore at 72 hours show absorption peaks near 290, 340, 460 and 490 nm, suggesting a carotenoid. Microspectrophotometry of isolated crystals also show similar absorption peaks (Figure 6).

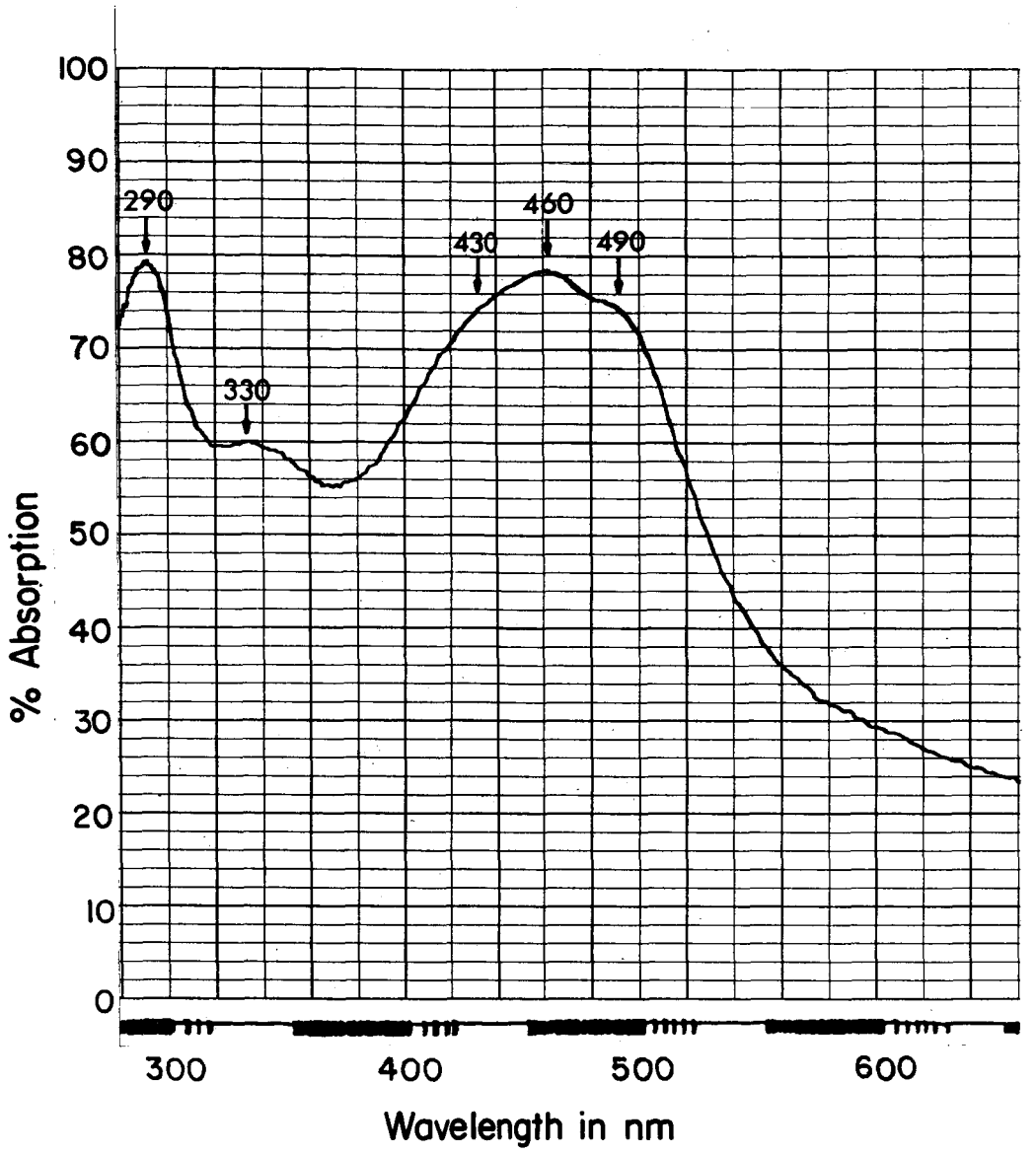


FIGURE 6. Spectrum of isolated crystals (Figure 5a) obtained from Phycomyces stage IVb.

c. Optical Diffraction and Symmetry of Phycomyces Crystals.

R. S. Morgan and J. J. Wolken.

Optical diffraction, like x-ray diffraction, is a technique for revealing structures. Examination of electron micrographs permits one to derive the three dimensions and cell parameters of crystalline inclusions from analysis of their optical diffraction patterns (Figures 7, 8 and 9).

Electron micrographs containing five different sections through the crystals of Phycomyces blakesleeanus (Figure 7) were found to be of two types. One type showed two sets of parallel lines at an angle of 80° to each other, while the other type showed only one set of parallel lines.

In attempting to visualize these two predominant types of images as two-dimensional projections of a three-dimensional structure, we were led to the assumption that the structure of these crystals consisted of parallel layers, or sheets, of elongated molecules, that the "criss-cross" images are projections normal to these layers, and that the "parallel" images are projections along the plane of these layers (Table IV). These assumptions, and a study of the diffraction patterns (Figure 8), brought us to the unit cell as shown in Figure 9.

We noted that all the "parallel" images so far seen in Phycomyces crystals correspond to views along the diagonal of the cell, as the periodicities along the lines are either 207 A (B_1) or 414 A (B_2). The cell is monoclinic and belongs to space group $P2_1$, with $\beta = 80°$, but the sides of the cell, although of similar lengths, appear to be unequal: a = 250 A, b = 280 A, c = 240 A (Figure 9).

TABLE IV

Phycomyces

Image	Type of Pattern	Plate Number	Angle in degrees	Spacings, Å, between lines	
"Criss-Cross"	A	1075	83	121	134
		1069	79	132	143
"Parallel"	B_1	1071	88	119	
		1069	90	122	
	B_2	1135	87	114	

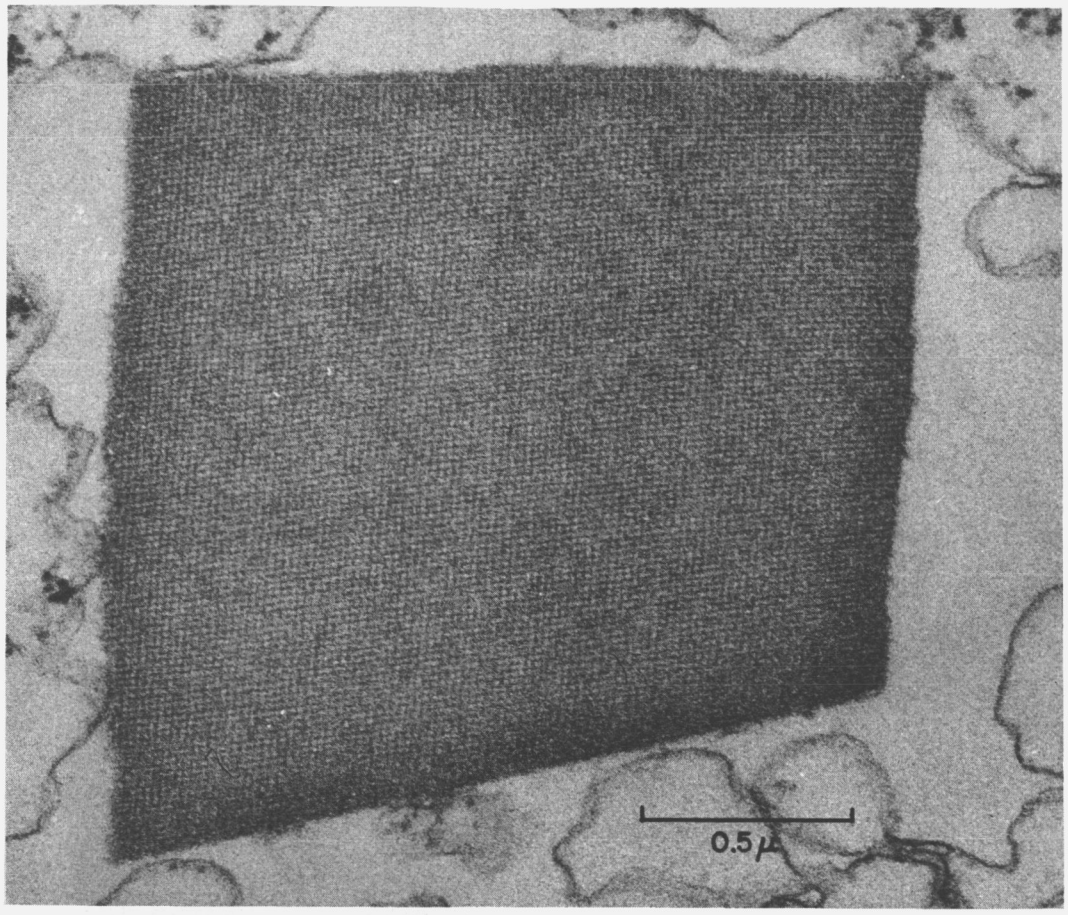


FIGURE 7. Electronmicrograph of a single Phycomyces crystal.

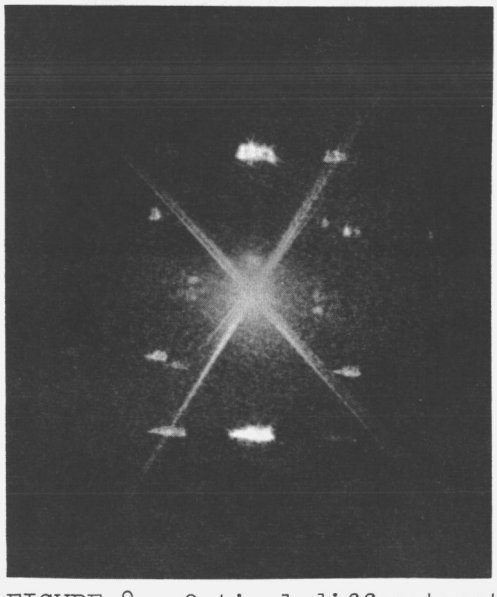


FIGURE 8. Optical diffractometer pattern from electron microscope plate of Phycomyces crystal.

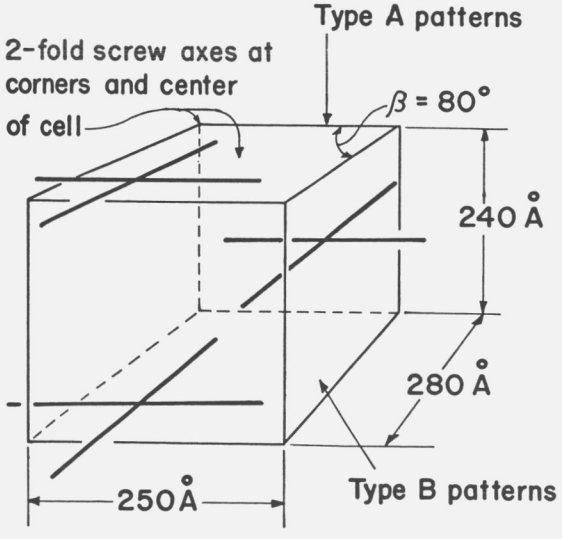


FIGURE 9. Three-dimensional model based on diffractometer pattern.

It was shown (Table II) that the isolated Phycomyces crystals have catalase activity. Therefore, it was of interest to compare the structure of these crystals with that of catalase purified from mammalian liver. Liver catalase is capable of forming several types of crystalline forms. The separated molecules of this protein have a molecular weight of 240,000, and consist of 4 equal subunits, each of dimensions 40 x 80 A. The Phycomyces crystals may be more complex, both in composition and in structure, than the crystals of purified catalase. Nonetheless, it is of interest that they also apparently contain 4 subunits in the 50,000 $\pm$ 10,000 range with a molecular weight of about 200,000.

B. Studies of the Visual Process

1. Arthropod Compound Eyes

The arthropods comprise the major group of invertebrate animals including insects, arachnids and crustacea, all of which possess image-forming compound eyes.

The compound eye consists of eye facets called ommatidia which vary in number from a few to as many as thousands. Each ommatidium has a corneal lens, crystalline cone and retinula cells. The retinula cells have specialized structures, rhabdomeres, which are analogous in function to the retinal rods and form the rhabdom, its retina.

In our previous Biophysical Research Laboratory Annual Reports, II-XIV, 1955-1969, we have summarized our investigations on the behavior, optical systems, structure and photopigments of compound eyes in search for understanding of the visual process. Here we would like to report additional studies of the firefly and carpenter ant.

a. Eye Structure

(1) The Firefly Eye. G. E. Marak, G. J. Gallik and J. J. Wolken.

The compound eye of the firefly (Photuris pennsylvanica) is structurally unique among insect eyes. Each ommatidium has a corneal lens, crystalline cone, and retinula cells. The ommatidium can be divided into two functional systems, the dioptric or optical system and the rhabdom or photoreceptor system. The firefly eye is called an exocone type because the multilayered lens has a conical extension into the region normally occupied by the crystalline cone in other insects (Figure 10). This unusual dioptric system has played an important role in theories of optical imaging in compound eyes. The structure of the rhabdom (Figure 11) has important implications for intraocular models of polarization analysis.

(2) The Carpenter Ant.

In the carpenter ant, Camponatus herculenus pennsylvanicus, the compound eye structure appears to represent a transition from the

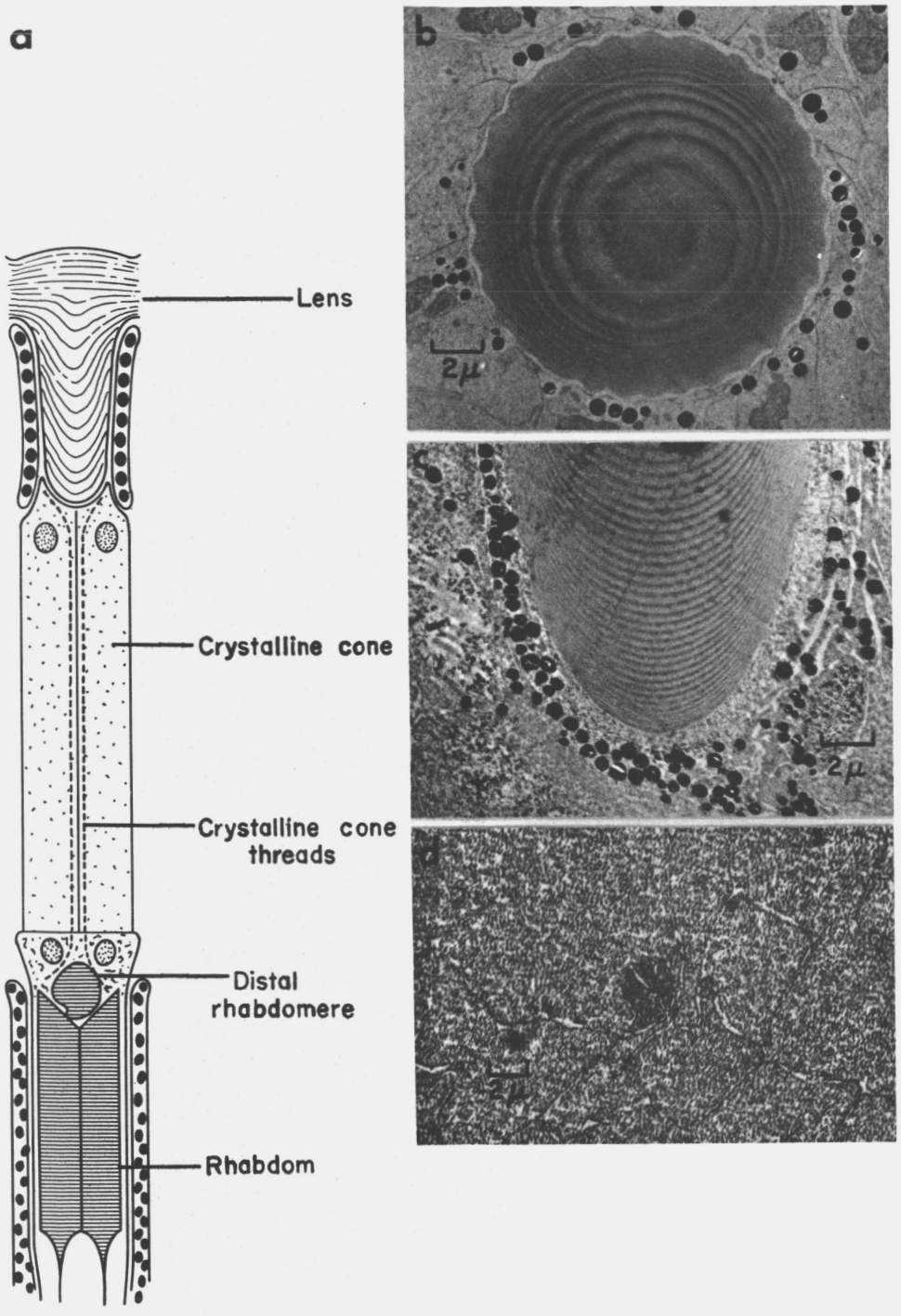


FIGURE 10. a, structure of the firefly ommatidium. b and c, exocone lens. d, crystalline cone threads. Photuris pennsylvanica.

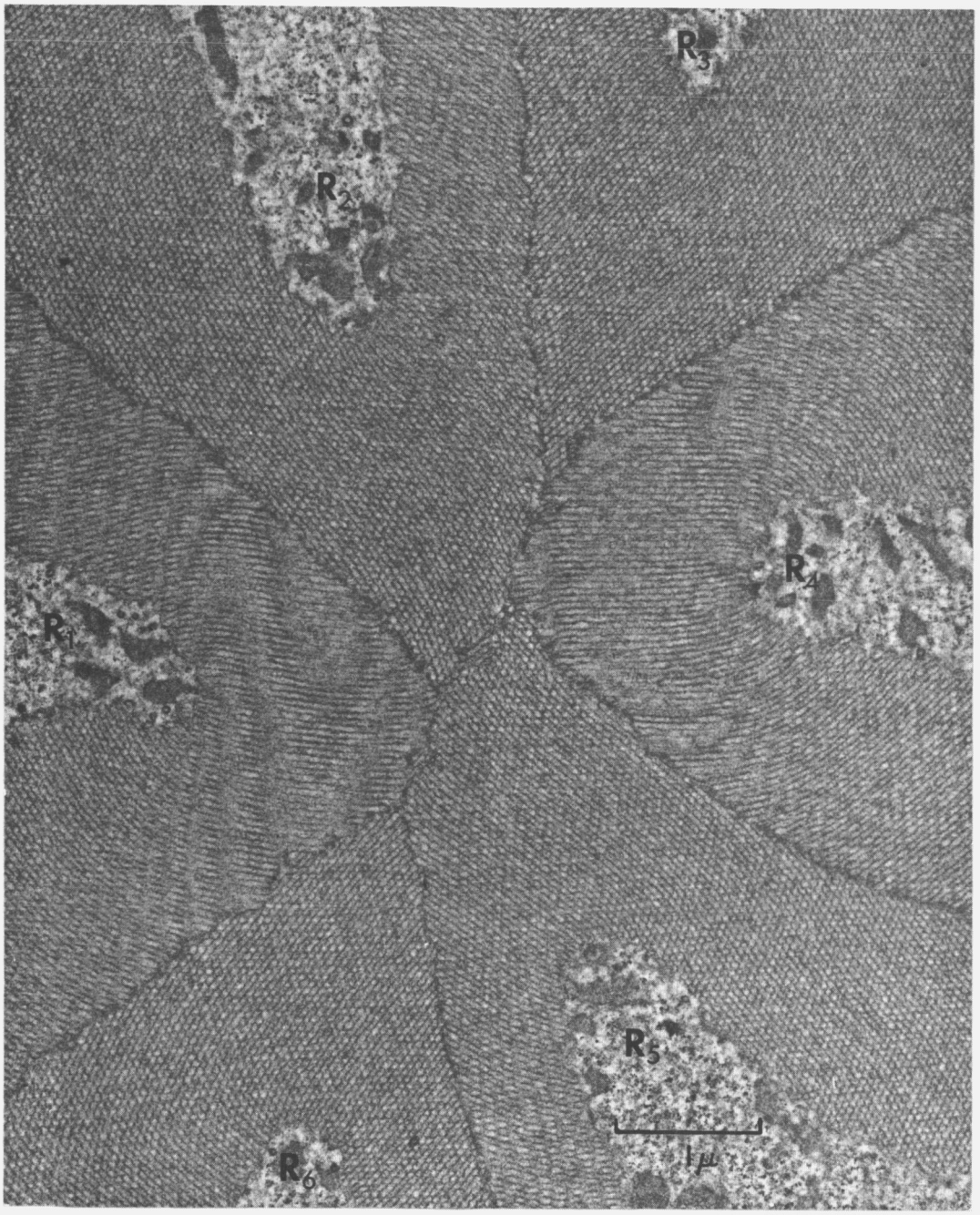


FIGURE 11. Rhabdom of firefly (Photuris pennsylvanica), enlargement of fine structure of rhabdomeres.

apposition type eye to that of the pseudocone described for the firefly (Figure 10) and the crystalline thread of the hornet.

The retinula cells of the carpenter ant show two zones, a clear zone near the rhabdom and a peripheral cytoplasmic zone. The rhabdom (Figures 12 and 13) of the carpenter ant is circular and occupies a much larger portion of the ommatidial cross-section than in the hornet. Despite its size, the rhabdom is formed from only six retinula cells as compared to eight or nine in other insects.

The microtubules of the rhabdomere have an elaborate fine structure. There appears to be a lobulated intratubular material at the center of which is a star-shaped electron dense body (Figure 14). At the angles of adjacent microtubules are found electron dense intervillous bridges. These intervillous bridges bind adjacent microtubules.

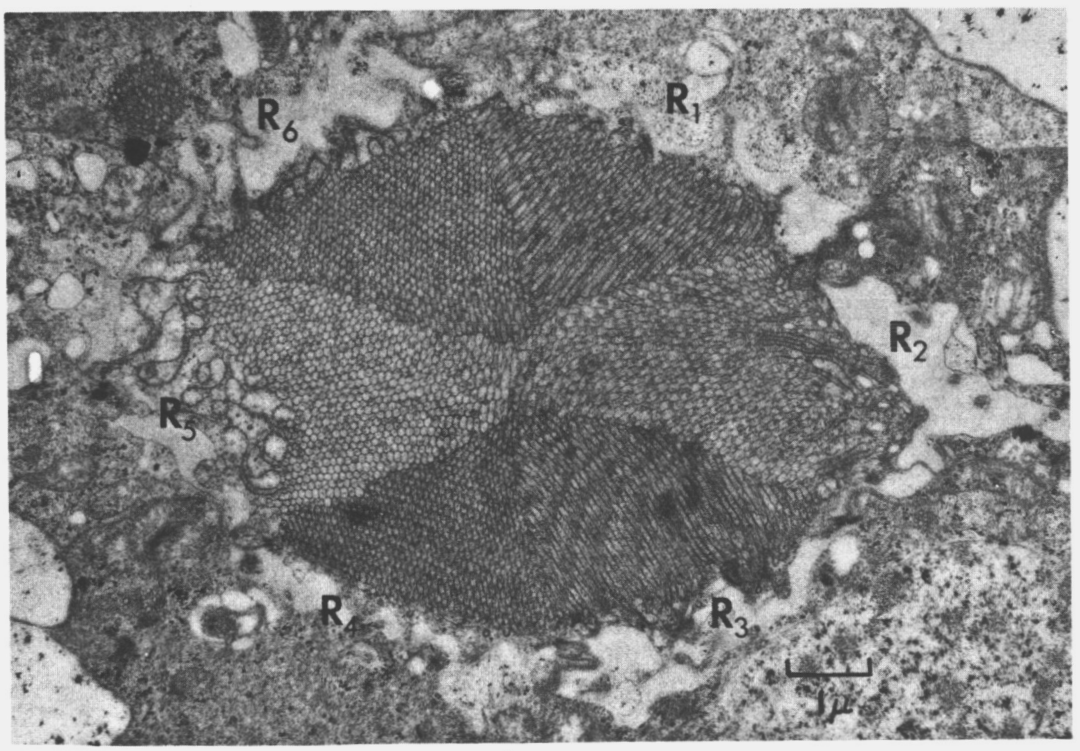


FIGURE 12. The carpenter ant (Camponotus herculenus pennsylvanicus), cross-section through the rhabdom showing its rhabdomeres (R_1-R_6).

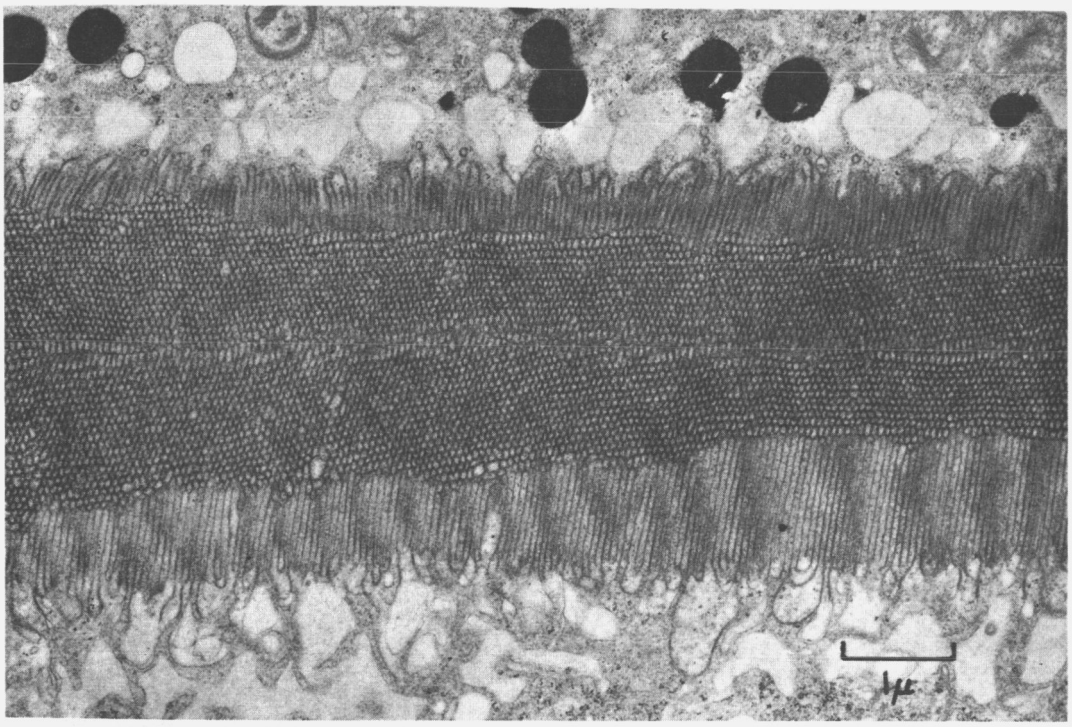


FIGURE 13. The carpenter ant (Camponotus herculenus pennsylvanicus), longitudinal section of a rhabdom.

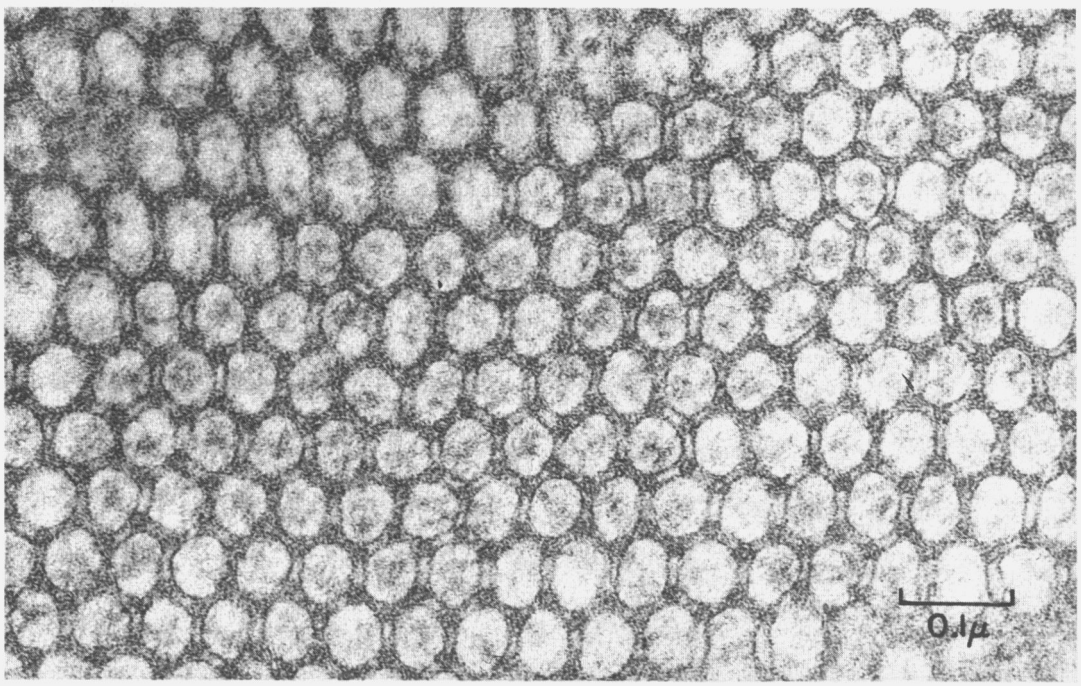


FIGURE 14. High resolution electron micrograph of a cross-section through many rhabdomere microtubules.

b. Invertebrate Visual Pigments

What are the invertebrate visual pigments? The invertebrates present a diverse group of animals, and equally diverse photoreceptors -- from eyespots, ocelli compound eyes to refracting eyes (Figure 18). Even though too few visual pigments have been isolated and their photochemistry studied, we can try to piece together the experimental data obtained from the extracted visual pigments, behavior, electrophysiology and microspectrophotometry.

In the search to identify the visual pigments of invertebrates, it was anticipated that they would not be too different from the vertebrates. All the vertebrate visual pigments identified so far are complexes in which the chromophore 11-cis retinal is attached to a specific protein opsin to form a rhodopsin. The important step, then, for visual excitation is that upon irradiation by light, rhodopsin liberates the all-trans retinal from opsin.

We can now turn to the invertebrate to see if there is a universal pigment chemistry for all visual systems.

The visual pigment of invertebrates is also a rhodopsin in which the chromophore is retinal (Figure 15). Cephalopod and crustacean rhodopsin light bleaching involves only the transformation to metarhodopsin; the liberation of free retinal and opsin does not occur as in vertebrate rhodopsin.

Spectral sensitivity measurements indicate that there are several absorbing pigments and that like the rods and cones of vertebrate eyes these are used for bright and dim light and for color discrimination. For example, the lobster visual pigment shows absorption peaks at 515 and 480 nm, the crayfish at 562 and 510, the honeybee at 430 and 530, and the housefly at 437 and 510 nm. In addition, there is an absorption peak in the neighborhood of 340 to 390 nm.

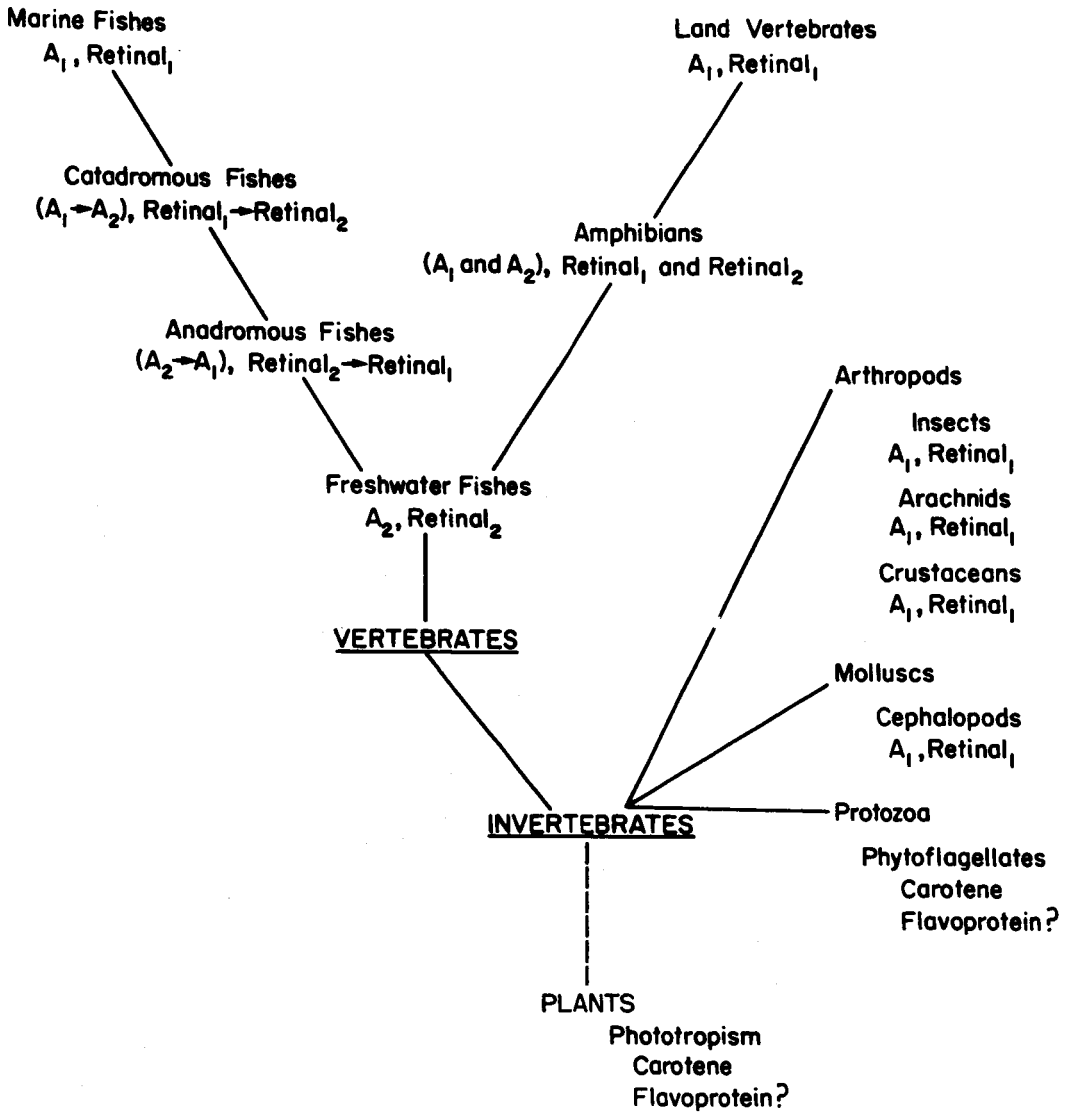


FIGURE 15. Carotenoid pigments associated with photoreceptors.

c. Screening Pigments

Invertebrate eyes also possess screening and reflecting pigment granules which serve to regulate the amount of light that reaches the photoreceptors, the rhabdom. They are ommochrome or pteridine pigments. What function these pigment granules may have in visual excitation is not completely known.

Ommochrome pigments are yellow to dark-red and fall into the class of ommatines and ommines. The ommochromes of the xanthommatin type are photosensitive, they can be oxidized and reduced, and are pH sensitive. The spectrum of xanthommatin is shown in Figure 16. It was suggested that these ommochromes could function as do the quinones with the cytochromes in the electron transfer chain. The pterine pigments are usually chemically represented as yellow xanthopterin (2-amino-4-6-dihydroxy pyrimido-pyrazine ring) which upon ultraviolet excitation, fluoresces blue. The pterines are stable in vivo but photosensitive in vitro. Although they take part in some metabolic processes in the eye, they do not appear to be part of the primary photoreceptor molecule in visual excitation.

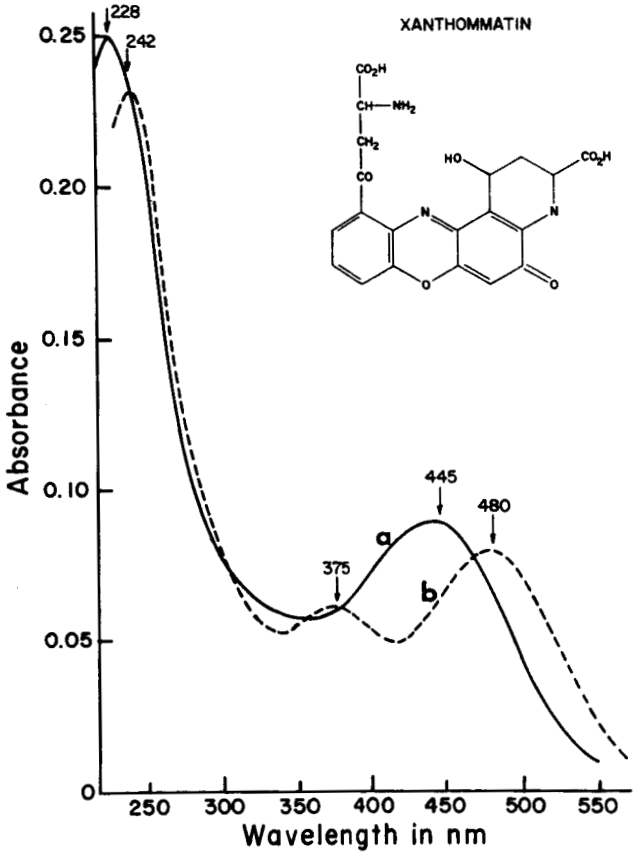


FIGURE 16. Absorption spectrum, xanthommatin, a) pH 7 and b) pH 2, 5N HCl.

Experimental evidence indicates that the screening pigments may function to absorb light for the visual pigment.

These studies of the invertebrate photoreceptors have been recently summarized in a monograph, INVERTEBRATE PHOTORECEPTORS: A Comparative Analysis, in press for publication in 1970 by Academic Press, Inc., New York.

2. Vertebrate Photoreceptors

Our studies of vertebrate photoreceptors during the past year were directed to the structure of the dog fish retina. The dog fish is not only of interest from a phylogenetic point of view, but also, too little is known of the visual system of elasmobranchs.

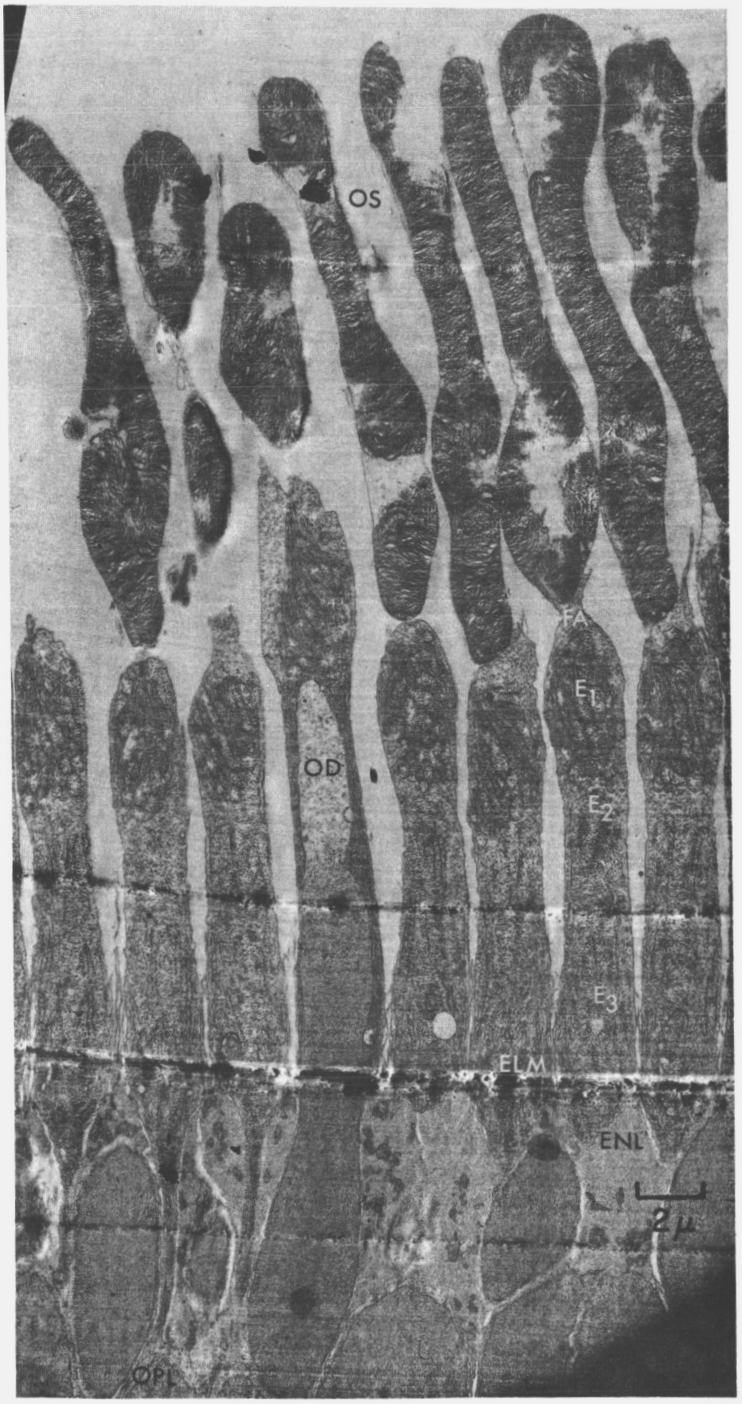
a. Structure of the Retina of the Smooth Dog Fish. M. A. Ali.

Dog fish (Mustelus canis) were obtained by J. J. Wolken and A. J. Wolken at the Marine Biological Laboratory (Summer of 1968), Woods Hole, Mass. The retinas were removed from the freshly enucleated eyes and these were fixed in 1% buffered osmium tetroxide, rinsed in sea water, and dehydrated through a series of graded alcohols to absolute. They were then embedded in Vestapol. Sections were cut from the polymerized blocks on a microtome and some sections were stained with uranyl acetate and others were counterstained with lead acetate. All sections were examined with a Philips 200 electron microscope.

The retina consists of ten cell layers which can be distinguished in the electron micrograph (Figure 17). The visual cells are fairly uniform and show no differentiation into what we can distinguish as rods and cones. The outer segment averaged 1.5 μ in diameter, and like vertebrate rods and cones is structured of double membrane discs about 140 Å in thickness; each membrane is about 40 Å in thickness. The double membraned discs are separated by a clear space of about 100 Å.

The visual cells are fairly uniform in appearance, although their dimensions vary from region to region. For example, the diameter varies from 1.4 to 2.3 μ and the length from 35 to 42 μ. In some sections it is possible to identify the outer segment, ellipsoid and the nucleus of the same cell. In these cases, the total length is about 40 μ. The rest of the cell is made of the visual cell fiber and the visual cell body. It extends from the lower end of the cell proper to the external plexiform layer. The visual cell fiber is a protoplasmic thread of smooth appearance and is about 1 μ in diameter.

The outer segments are attached to ellipsoids. Thus each visual cell consists of an outer segment and an ellipsoid. In the living state the outer segment is a smooth cylinder of uniform thickness with a rounded distal end. Its substance has been observed to have a peculiar brilliancy and to be homogeneous and positively birefringent.



OS Outer segment
FA Fiber apparatus
OD Oil droplet
E_1 Distal third ellipsoid
E_2 Middle third ellipsoid
E_3 Proximal third ellipsoid
ELM External limiting membrane
ENL External nuclear layer
OPL Outer plexiform layer

FIGURE 17. Electronmicrograph of smooth dog fish retina (Mustelus canis), showing outer and inner segments of rods.

The ellipsoids may be seen to contain three different types of regions. The distal third, adjacent to the fiber apparatus, is packed with long mitochondria, and a bundle of 15 to 18 fibrils running in one side of the outer segment and joining it to the inner segment of the rod. The middle third is granular while the proximal third is fibrous and vacuolar. The last region is probably analogous to the contractile myoid, found in teleosts and amphibians. It is also this region which passes through the external limiting membrane. In some ellipsoids, the middle and proximal thirds contain what appears to be an oil droplet.

When illuminated, the ellipsoid lengthens and pushes the outer segment deeper into the pigment epithelium toward the sclera. In dim light this region contracts, moving the outer segment in closer to the external limiting membrane and thus exposing it to more light. Little is known of the contractility of the myoid (proximal third of the ellipsoid) in the smooth dog fish or in the elasmobranchs.

It appears that the outer segments and ellipdoids of the elasmobranchs undifferentiated visual cells differ in their structure from the rods and cones of other vertebrates while the inner layers of the retina resemble those of vertebrates. This lends support to the view that the undifferentiated visual cells of the shark may be capable of functioning as do rods and cones.

The visual cell proper with the outer fiber is the homologue of the receptive dendritic expansions of a neuron. The horizontal cells are typical neurons whose bodies form the uppermost one or two rows of the internal nuclear layer. From the upper end of the body arise short dendritic twigs producing several tufts spreading in the lower zone of the external plexiform layer. The axis cylinder takes a horizontal course chiefly in the inner part of the external plexiform layer, and its terminal twigs synapse with the visual cell spherules. The horizontal cells thus receive impulses from a group of visual cells of one locality and transmit them to a group of visual cells of another locality.

The bipolar cells connect the visual cells with the ganglion cell of the retina, and through these with the visual centers of the brain via the optic nerve fibers. The bipolar cells stand approximately upright with respect to the retinal layers. Their nuclei are in the internal nucelar layer and the external plexiform layer -- with very few in the latter. Each bipolar has one or several outward expansions that spread into the external plexiform layer, where they synapse with the visual cells. It is interesting to see the intricate manner in which the dendritic expansions of the bipolar cells penetrate the terminal swellings of the visual cells. A single inward expansion of the bipolar spreads into the internal plexiform layer where it is synaptically related to the ganglion cells and other adjoining cells. Two groups of bipolars can be distinguished -- centripetal bipolars, which transmit impulses from the visual cells to the ganglion cells and centrifugal bipolars, which transmit in the opposite direction.

The ganglion cells represent the third relay, in the retina, of the chain of neurons that form the afferent visual pathway. These cells are larger than those of the two nuclear layers, and closely resemble neurons of the brain. The bodies are in the ganglion layer, with a few displaced into the lower part of the internal plexiform layer. Their dendrites spread in the inner plexiform layer. Chromophile substance is present in all. From the perikaryon or the chief dendrite trunk of each ganglion cell arises one axis cylinder that leaves the retina and becomes an optic nerve fiber terminating in the optic tectum of the brain.

The retina contains supporting elements of neuroglial character. The most important are the radial fibers of Müeller. These are present throughout the retina in all regions. Their oval nuclei lie in the middle zone of the inner nuclear layer. The cell body is a slender fiber or pillar which extends radially from the external to the internal limiting membrane. Their inner ends expand conically and form the internal limiting membrane. In the two plexiform layers

the radial fibers give off many branches, which form a dense neuroglial network.

The cell bodies of the radial fibers are beset with excavations which envelop bodies of the ganglion cells, bipolars, horizontal cells and the visual cells. The bodies of the nervous elements appear to be completely enveloped in thin husks of supporting structures which perhaps serve as insulators. The visual cell fibers are likewise encased in thin tubelike sheaths produced by Müeller's fibers.

At the limit of the external nuclear layer and visual cell layer the radial fibers fuse and form the external limiting membrane.

The retina of the smooth dog fish in organization has attained a very high level of development comparable to that in teleosts and mammals.

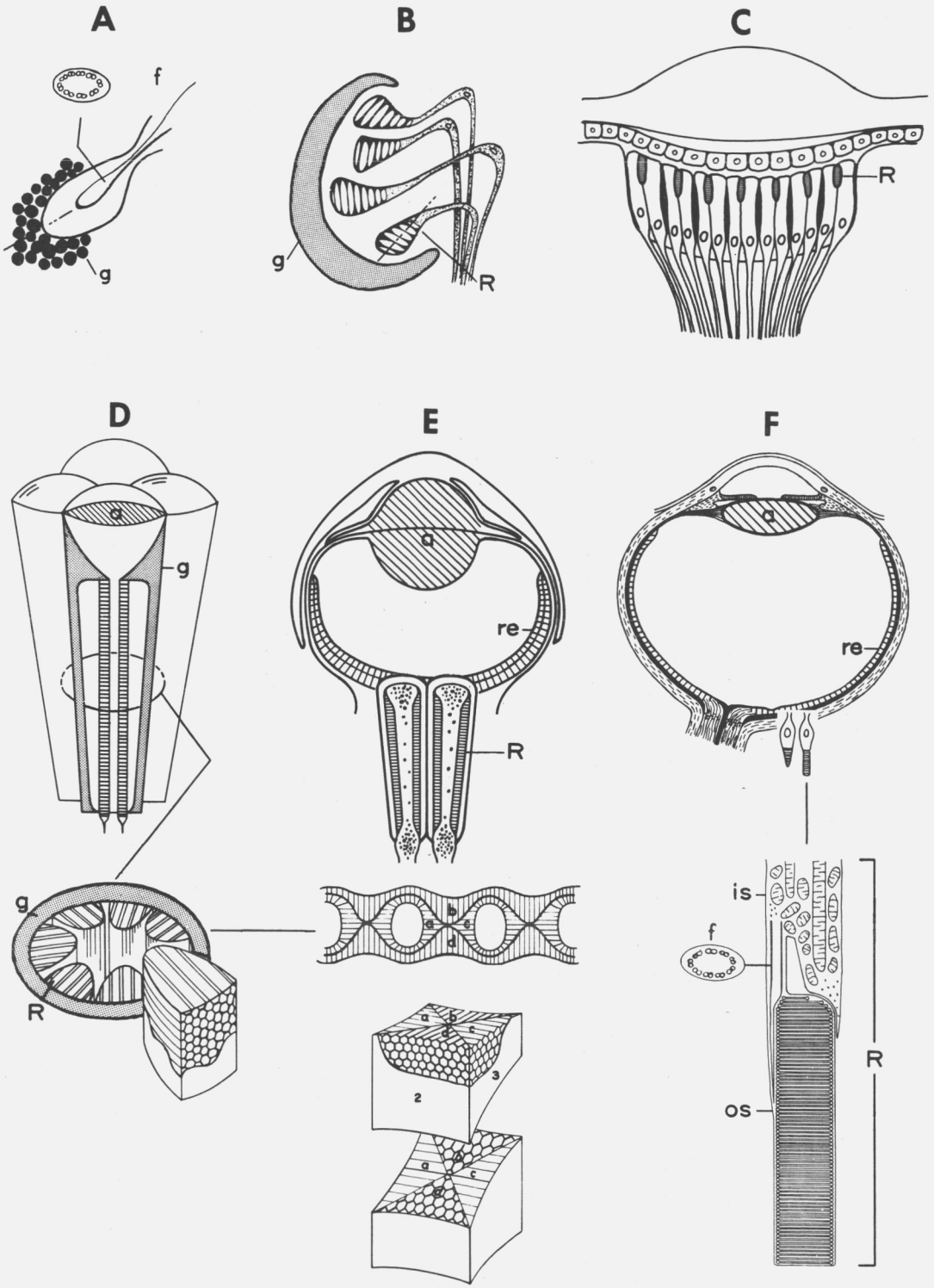


FIGURE 18. Schematic structure of various kinds of eyes and their photoreceptors. A, Eyespot of protozoa; B, Photosensory cell of the flatworm ocellus; C, Insect ocellus; D, Compound eye of arthropods; E, Mollusc cephalopod eye; F, Vertebrate eye; a, lens, f, flagellum, g, pigment, R, photoreceptor (retinal rod or rhabdomere) os, outer segment, is, inner segment, re, retina.

C. Instrumentation

1. Microspectrophotometer. J. J. Wolken.

The construction of recording microspectrophotometers of various designs has developed rapidly in the past decade. We have designed and constructed over the past eight years microspectrophotometers M-1 through M-5. These instruments were immediately applied to a variety of biological, biochemical and chemical studies.

The main characteristics of the recording microspectrophotometer M-5 are improved optical resolution and greater sensitivity from the ultraviolet through the visible to the infrared. Spectra can be obtained from minute drops of solution and from particles as small as 0.5 μ in diameter. The efficiency of the instrument is dependent upon the novel design of the chopper and the associated electronics. Continuous spectra can be recorded from the ultraviolet, 1800 Å to 1 μ in the infrared. The visible part of the spectrum, 4000 to 8000 Å is recorded within one second.

The present instrument is versatile, rugged and adaptable to performing a variety of spectroscopic analyses from living cells, cell organelles, particles and solutions, which would be difficult to obtain by other methods.

The optical and electronic design of the microspectrophotometer is summarized in the publication, Rev. Sci. Instrum. 39:1734, 1968 .

Studies during the past year were directed toward the biosynthesis and identification of pigment molecules; for example, porphyrins, chlorophylls, carotenes and flavins in living cells. In addition, spectral analysis of blood hemoglobins are being obtained to identify these hemoglobins in the blood cells of healthy and diseased states.

Also, several interesting applications were carried out in collaboration with Professors Sidney Fox and George Mueller, Institute of Molecular Evolution, University of Miami, Miami, Florida. One has to do with the chemistry of laboratory synthesized microspheres, believed to be a precursor structural model for a living cell. The others have to do with geochemical (from samples of foxite, ozocenite, quartz inclusion bodies, mica and other minerals) and their possible

relationship to the origin of life.

Finally, and of great interest, were lunar samples from the Apollo 11 Mission. The lunar material consisted of small "glass" spheroids, which ranged in size from 0.1 to 500 μ in diameter. The analysis by microspectrophotometry indicated difference in their absorption characteristics.

In almost all particles, there was strong ultraviolet absorption. The result of these analyses of lunar material is published by S. W. Fox, K. Harada, P. E. Hare, G. Hinsch and G. Mueller, Bio-organic Compounds and Glassy Microparticles in Lunar Fines and Other Materials, Science 167:767, 1970.

2. Optical Diffractometer. J. J. Wolken and O. J. Bashor.

We have already indicated the use of optical diffraction (pages 20 and 21 and Figures 7, 8 and 9), as a technique for examining structure revealed by electron microscopy (Figure 7). Optical diffraction, like x-ray diffraction, permits one to reconstruct the molecular structure and estimate the molecular weight of these crystalline cellular inclusions.

We are constructing an Optical Diffractometer, modeled after the design of Berger and Harker (J. E. Berger and H. Harker, Optical Diffractometer for Production of Fourier Transforms of Electron Micrographs, Rev. Sci. Instrum. 38:292, 1966).

The basic design of this instrument is illustrated in Figure 19. The lenses are two planoconvex lenses with a focal length of 1.52 meters with a surface finish of 0.1 wavelength. The light source is an Edmund Continuous wave HE-NE Laser with an emitting light of 632.8 nm.

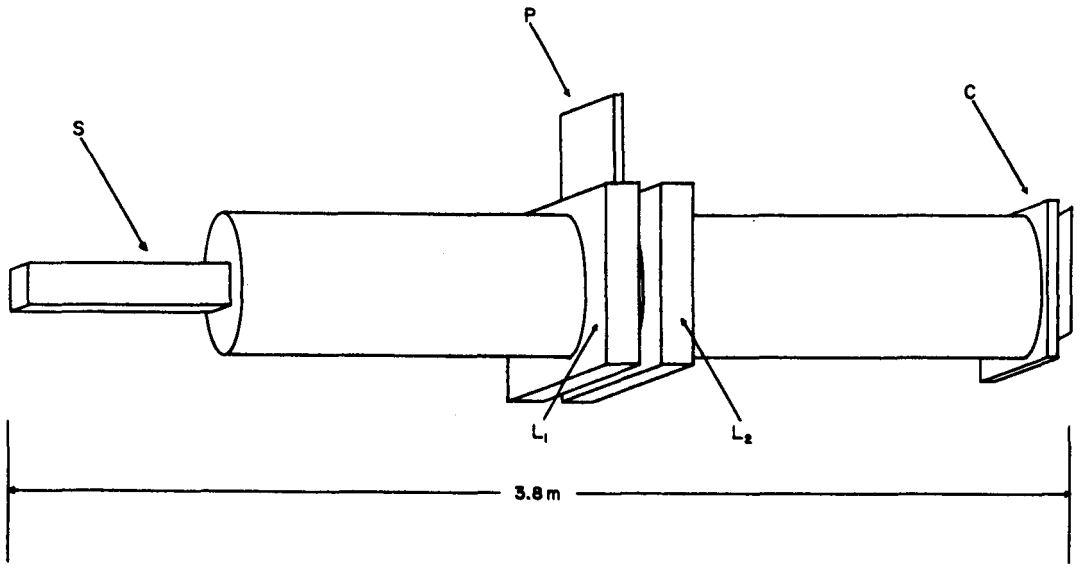


FIGURE 19. Schematic of optical diffractometer constructed in the Biophysical Research Laboratory. S, Laser, P, Plate holder, L_1, Fixed lens holder, L_2, Adjustable lens holder, C, Film holder.

3. Electromagnetophoresis. A. Perlin and E. L. Morofsky.

A study was initiated to determine whether human red blood cells of normal and diseased states behave differently when exposed to combined magnetic and electric fields.

Electromagnetophoresis is applicable to the separation of particles of nearly equal density but distinctly different electrical conductivity. In this case, by adjusting the conductivity of the surrounding fluid to an intermediate value, the two species can be made to migrate in opposite directions. Particles of different shapes may be separated even when their densities, volumes, and electrical conductivity of irregular bodies and of microscopic particles may be measured by finding the conductivity of the solution in which they experience no electromagnetic force. In this way, the measurement of the electrical conductivity of various living cells is possible. Alternating currents and fields must be used to avoid electrical stimulation of the cells. Such measurements of variation in the electrical conductivity of active cells are of interest in our studies of changes in cell membrane permeability in response to stimuli.

The apparatus consists of a quartz migration cell fitted with platinum electrodes on two opposite faces, placed between the poles of a large electromagnet. Whole blood or washed cells were used. After migration of the red blood cells, the quartz cell is placed in a spectrophotometer modified to scan the sample along the direction of migration giving the distribution of light transmission as a function of distance from the bottom of the cell for various wavelengths.

The major difficulty encountered was the construction of a migration cell that would meet requirements both for spectrophotometry and electromagnetophoresis. Since very high magnetic fields were required, large amounts of heat are released, therefore, the dimensions of the cell are very critical. In the experimental runs to date, these currents were found to invalidate the results obtained.

VI. RESEARCH, PUBLICATIONS AND REPORTS

A. Publications

Wolken, J. J. and R. G. Florida. 1969. The Eye Structure and Optical System of the Crustacean Copepod, Copilia. J. Cell Biol. 40:279.

Wolken, J. J. 1969. Photoreceptor Molecules and the Phototactic Response of Euglena. J. Cell Biol. 43:159a.

Wolken, J. J. 1969. Microspectrophotometry and the Photoreceptor of Phycomyces I. J. Cell Biol. 43:354.

Wolken, J. J. 1969. In Search of the Photoreceptor System of Phycomyces. J. Cell Biol. 43:354.

Wolken, J. J. 1970. The Eye: The Retina, In The Encyclopedia of Biological Sciences. P. Gray, ed. (2nd revised edition), van Nostrand Reinhold Publishing Company, New York. pp. 322-325.

Wolken, J. J. 1970. Cell and Photoreceptor Membranes, In Physical Principles of Biological Membranes. F. Snell, J. J. Wolken, G. J. Iverson and J. Lam, eds. Gordon and Breach Science Publishers, New York. pp. 365-382.

Wolken, J. J. Photodynamics: The Chloroplast, In Phytochemistry. L. P. Miller, ed. van Nostrand Reinhold Publishing Company, New York (in press for 1970).

Morgan, R. S., A. R. Czeto, S. E. Frederick, E. H. Newcomb and J. J. Wolken. 1970. Crystal Symmetry of Nicotiana tabacum and Phycomyces blakesleeanus Have the Same Symmetry (submitted for press).

Refer to Annual Reports I-XIV, 1953-1969, for other publications; reprints of published papers, when available, will be sent upon request.

B. Reports and Participation in Scientific Meetings

Wolken, J. J. February 28 - March 1, 1969. In Search of the Photoreceptor System of Phycomyces. THIRTEENTH ANNUAL MEETING OF THE BIOPHYSICAL SOCIETY. Los Angeles, California.

Marak, G. E. March 7, 1969. ASSOCIATION FOR RESEARCH IN OPHTHALMOLOGY: INSECT VISUAL PIGMENTS. Atlantic Section Meeting. Washington, D.C.

Wolken, J. J. March 12, 1969. NATIONAL HIGH SCHOOL LECTURE, Dade County. Light and Molecular Biology, UNIVERSITY OF MIAMI. Miami, Florida.

Wolken, J. J. August 20 - 22, 1969. The Photoreceptor Molecule and the Phototactic Responses of Euglena. SYMPOSIUM OF TAXES AND KINESIS OF FLAGELLATED CELLS. University of California. Santa Barbara, California.

Wolken, J. J. August 24 - September 2, 1969. The Photoreceptor System of Phycomyces. XI INTERNATIONAL BOTANICAL CONGRESS, University of Washington. Seattle, Washington.

Wolken, J. J. November 6 - 8, 1969. The Photoreceptor Molecule and the Phototactic Response of Euglena. AMERICAN SOCIETY FOR CELL BIOLOGY. Detroit, Michigan.

Wolken, J. J. December 4, 1969. What We Can Learn From the Retina. PITTSBURGH NEURAL SCIENCE LECTURE SERIES, University of Pittsburgh School of Medicine. Pittsburgh, Pennsylvania.

Gallik, G. J., P. J. Dubash and J. J. Wolken. February 25 - 27, 1970. Isolation of Crystals from Phycomyces. XIV ANNUAL MEETING OF BIOPHYSICAL SOCIETY. Baltimore, Maryland.

Wolken, J. J. April 14 - 17, 1970. FIFTY-FOURTH ANNUAL MEETING OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY. Atlantic City, New Jersey.

C. Books Published by Jerome J. Wolken

EUGLENA: AN EXPERIMENTAL ORGANISM FOR BIOCHEMICAL AND BIOPHYSICAL STUDIES. 1961. The Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey.

VISION: BIOPHYSICS AND BIOCHEMISTRY OF THE RETINAL PHOTORECEPTORS. 1966. Charles C. Thomas, Publisher, Springfield, Illinois.

EUGLENA: AN EXPERIMENTAL ORGANISM FOR BIOCHEMICAL AND BIOPHYSICAL STUDIES, SECOND EDITION. 1967. Appleton-Century-Crofts, New York.

PHOTOBIOLOGY. 1967. Reinhold Publishing Company, New York.

INVERTEBRATE PHOTORECEPTORS. Academic Press, Inc., New York (in press for 1970).

Edited Works

PHOTORECEPTION. 1958. J. J. Wolken, ed. Annals of New York Academy of Sciences, 74:161-406.

PHYSICAL PRINCIPLES OF BIOLOGICAL MEMBRANES. 1970. F. Snell, J. J. Wolken, G. J. Iverson and J. Lam, eds., Gordon and Breach Science Publishers, New York.

VII. ACKNOWLEDGEMENTS

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June 30, 1970
DATE

Jerome J. Wolken